

1       **METHOD AND PHARMACEUTICAL COMPOSITION FOR THE**  
2                               **TREATMENT OF IMMUNE DISORDERS**

3       BACKGROUND OF THE INVENTION

4       1. Field of the Invention

5               The present invention relates generally to the treatment of immune  
6 disorders and more specifically to suppress the levels of T-cell activation and  
7 proliferation in immune disorders. More particularly, the present invention  
8 provides a pharmaceutical composition and a method of using CD30 or a  
9 biologically functional equivalent thereof to suppress the levels of T-cell  
10 activation and proliferation in immune disorders.

11      2. Description of Related Art

12             Hodgkin's disease (HD) is characterized by numerous infiltrating  
13 immune cells, especially T cells, surrounding malignant cells, which comprise  
14 less than one percent of the total cell population in the tumor (Cossman et al.,  
15 *Lab. Invest.* 78:229,1998). The tumor cells clearly succeed in evading attacks  
16 by these immune cells.

17             The neoplastic cells (i.e., Hodgkin and Reed-Sternberg, H-RS cells) in  
18 HD are characterized by an abundant expression of CD30, a member of the  
19 TNFR (tumor necrosis factor receptor) superfamily (Durkop et al., *Cell*, 68:421,  
20 1992). CD30 is a 120kd surface antigen widely used as a clinical marker for  
21 HD.

22             CD30 was originally thought to be a receptor that carried out its  
23 function through engagement with its respective ligand, CD30L (CD153)  
24 (Smith et al. *Cell*, 73:349, 1993), expressed as a type II membranc glycoprotein

1 in activated T cells, and other types of cells (Nicod et al., *Am. J. Resp. Cell.*  
2 *Mol. Biol.*, 17:91, 1997; Pinto et al., *Blood.*, 88:3299, 1996). However, recent  
3 studies have shown a bi-directional instead of uni-directional signal  
4 transduction after the CD30-CD30L engagement in T and B cells (Shanebeck  
5 et al., *Eur. J. Immunol.*, 25:2147, 1995; Wiley et al., *J. Immunol.*, 157:3635,  
6 1996). CD30L cross-linking has been shown to induce gene expression and  
7 metabolic activity in human T cells and neutrophils. Engagement of CD30L by  
8 CD30 on T cells inhibits class switching as well as IgG, IgA, and IgE  
9 production in B cells (Cerutti et al., *Nat. Immunol.*, 2:150, 2001; Cerutti et al., *J.*  
10 *Immunol.*, 165:786, 2000). A viral counterpart of CD30 has also been shown to  
11 function in a similar fashion. (Saraiva et al., *J. Exp. Med.*, 196: 829, 2002.)

12 The role of CD30-CD30L interaction in health and disease is still not  
13 totally understood. Potential therapeutic uses for CD30 or a biologically  
14 functional equivalent thereof have yet to be identified.

15 SUMMARY OF THE INVENTION

16 An objective of the present invention is to provide a method for  
17 treating immune disorders by lowering the levels of T-cell activation and  
18 proliferation by administering CD30 or a biologically functional equivalent  
19 thereof to a human afflicted with immune disorders.

20 The other objective of the present invention is to provide a  
21 pharmaceutical composition for treating immune disorders in a human  
22 comprising a therapeutically effective amount of CD30 or a biologically  
23 functional equivalent thereof, and a pharmaceutically acceptable carrier,  
24 excipient or diluent.

1 Further benefits and advantages of the present invention will become  
2 apparent after a careful reading of the detailed description with appropriate  
3 reference to the accompanying drawings.

#### 4 BRIEF DESCRIPTION OF THE DRAWINGS

5 Figs. 1A, 1B and 1C are schematic diagrams showing the construction  
6 of a recombinant soluble human CD30-Fc chimeric protein and show the  
7 monomeric structure of the recombinant human CD30-Fc fusion protein;

8 Fig. 2 shows the construction of plasmid pMIB rhu CD30-Fc;

9 Figs. 3A and 3B are graphs showing the inhibitory effect of either  
10 plate-bound or soluble CD30-Fc fusion protein on T-cell proliferation;

11 Fig. 4 is a graph showing the inhibitory effect of CD30-Fc fusion  
12 protein on the production of IL-2 by anti-CD3-stimulated T cells;

13 Fig. 5 is a graph showing the effect of IL-2 addition on T-cell  
14 proliferation in the presence of CD30-Fc fusion protein; and

15 Fig. 6 shows the effect of CD30-Fc fusion protein on the inhibition of  
16 CD25 and CD26 expression in anti-CD3-stimulated T cells.

#### 17 DETAILED DESCRIPTION OF THE INVENTION

##### 18 Definitions

19 As used herein, the term "CD30" refers to a protein having an amino  
20 acid sequence substantially similar to the amino acid sequence of the native  
21 mammalian CD30 protein, which are capable of binding to CD30 ligand  
22 molecules. In the absence of any species designation, CD30 refers generically  
23 to mammalian CD30. Similarly, in the absence of any specific designation, the  
24 term CD30 means all forms of CD30.

1           The term "biologically functional equivalent thereof," as used  
2 throughout the specification, means that the function of a substance described  
3 as "the biologically functional equivalent" is the same as the function of CD30.  
4 Preferably, the function of the biologically functional equivalent thereof is  
5 capable of binding to CD30 ligand and transmitting a stimulus to a cell. More  
6 preferably, the biologically functional equivalent within the scope of the  
7 present invention is capable of binding to CD30 ligand and inhibiting the levels  
8 of T cell proliferation and/or activation. Preferably, the biologically functional  
9 equivalent may be an analog, a subunit, a mutant, an agonist polypeptide, a  
10 chimeric protein or a derivative of CD30.

11           The term "purified," as used in the context of this specification to  
12 define the purity of CD30 protein or protein compositions, means that the  
13 protein or protein composition is substantially free of other proteins of natural  
14 or endogenous origin and contains less than about 1% by mass of protein  
15 contaminants residual of production processes. However, such compositions  
16 can contain other proteins added as stabilizers, carders, excipients or co-  
17 therapeutics.

18           The term "recombinant," as used herein, means that a protein is derived  
19 from recombinant (e.g., mammalian) expression systems. Protein expressed in  
20 insect cells may have a glycosylation pattern different from that expressed in  
21 mammalian cells.

22           The term "therapeutically effective amount," as used herein, means that  
23 an amount of CD30 or a biologically functional equivalent thereof is sufficient  
24 for an effective treatment when administered to a human in need of such

1 treatment. The therapeutically effective amount will vary depending upon the  
2 subject and disease condition being treated, the weight and age of the subject,  
3 the severity of the disease condition, the manner of administration and the like,  
4 which can readily be determined by one of ordinary skill in the art.

5 The term "pharmaceutically acceptable," as used herein, means that the  
6 carrier, diluent or excipient employed in the pharmaceutical composition  
7 according to the present invention must be compatible with the other  
8 ingredients of the composition and not deleterious to the recipient of the  
9 composition.

10 The term "locally," as used herein, means the CD30 or the biologically  
11 functional equivalent thereof is injected into a confined space in a human body,  
12 for example, a joint space, the pleural space, or an eye ball.

13

14 CD30 protein

15 The CD30 protein is not limited to a native CD30 protein but includes  
16 an artificial CD30 construct (e.g. genetically engineered CD30 construct). The  
17 native CD30 protein is a membrane protein. The mature full-length human  
18 CD30 is a glycoprotein having a molecular weight of about 110-120  
19 kilodaltons (kDa). The CD30 protein employed according to the present  
20 invention may have an amino acid sequence corresponding to all or part of the  
21 extracellular region of a native CD30 and are biologically active. Preferably,  
22 the CD30 protein is capable of binding to a CD30 ligand molecule. In a  
23 preferred embodiment of the present invention, the CD30 protein is in soluble  
24 form.

1 Induction of CD30 ligand signal transduction activity can be  
2 determined by transfecting cells with recombinant CD30 ligand DNAs to  
3 obtain recombinant CD30 ligand expression. The transfected cells are then  
4 placed in contact with the CD30 protein, and the resulting metabolic effects  
5 examined. If an effect results which is attributable to the action of the CD30,  
6 then the recombinant CD30 ligand has signal transduction activity. Exemplary  
7 procedures for determining whether a polypeptide has signal transduction  
8 activity are disclosed by Idzerda et al., *J. Exp. Med.*, 171:861 (1990); Curtis et  
9 al., *Proc. Natl. Acad. Sci. U.S.A.*, 86:3045 (1989); Prywes et al., *EMBO J.*,  
10 5:2179 (1986) and Chou et al., *J. Biol. Chem.*, 262:1842 (1987). Alternatively,  
11 primary cells or cell lines that express an endogenous CD30 ligand and have a  
12 detectable biological response to CD30 could also be utilized.

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#### 14 Biologically Functional Equivalent of CD30

15 The biologically functional equivalent of CD30 employed according to  
16 the present invention may have an amino acid sequence corresponding to all or  
17 part of the extracellular region of a native CD30 and are biologically active. In  
18 a preferred embodiment of the present invention, the biologically functional  
19 equivalent of CD30 is in soluble form. Preferably, the biologically functional  
20 equivalent may be an analog, a subunit, a mutant, an agonist polypeptide, a  
21 chimeric protein or a derivative of CD30.

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23 Analog or subunit of CD30

24 A soluble biologically functional equivalent of CD30 employed

1 according to the present invention may include, for example, analogs or  
2 subunits of a native CD30 protein having at least 20 amino acids and exhibit at  
3 least some biological activity in common with CD30. The soluble biologically  
4 functional equivalent of CD30 may be devoid of a transmembrane region and  
5 intracellular domain but retain the ability to bind a CD30 ligand. The soluble  
6 biologically functional equivalents of CD30 protein may include polypeptides,  
7 varying from the amino acid sequence of native CD30 by one or more  
8 substitutions, deletions or additions and retain the ability to bind to a CD30  
9 ligand and induce signal transduction activity via cell surface bound CD30  
10 ligand proteins.

11 Induction of CD30 ligand signal transduction activity can be  
12 determined by transfecting cells with recombinant CD30 ligand DNAs to  
13 obtain recombinant CD30 ligand expression. The transfected cells are then  
14 placed in contact with the biologically functional equivalent of CD30, and the  
15 resulting metabolic effects examined. If an effect results which is attributable to  
16 the action of the biologically functional equivalent of CD30, then the  
17 recombinant CD30 ligand has signal transduction activity. Exemplary  
18 procedures for determining whether a polypeptide has signal transduction  
19 activity are disclosed by Idzerda et al., *J. Exp. Med.*, 171:861 (1990); Curtis et  
20 al., *Proc. Natl. Acad. Sci. U.S.A.*, 86:3045 (1989); Prywes et al., *EMBO J.*,  
21 5:2179 (1986) and Chou et al., *J. Biol. Chem.*, 262:1842 (1987). Alternatively,  
22 primary cells or cell lines that express an endogenous CD30 ligand and have a  
23 detectable biological response to the biologically functional equivalent of  
24 CD30 could also be utilized.

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Agonist polypeptide of CD30

A biologically functional equivalent of CD30 employed according to the present invention may include purified CD30 agonist polypeptides. The purified CD30 agonist polypeptides used in this invention are substantially free of other contaminating materials of natural or endogenous origin and contain less than about 1% by mass of protein contaminants residual of production processes. The CD30 agonist polypeptides used in this invention are optionally modified with glycosylation.

Like most other mammalian proteins, the CD30 protein is encoded by a multi-exon gene. Alternative mRNA constructs that can be attributed to different mRNA splicing events following transcription and share large regions of identity or similarity with the cDNA first published (Durkop et al., *Cell*, 68:421,1992) may also be used.

Other mammalian CD30 cDNAs may be isolated by using appropriate human CD30 DNA sequences as a probe for screening a particular mammalian cDNA library by cross-species hybridization. Mammalian CD30 cDNAs can be obtained by cross species hybridization, using a single stranded cDNA derived from the human CD30 DNA sequence as a hybridization probe to isolate CD30 cDNAs from mammalian cDNA libraries.

Derivative of CD30

A biologically functional equivalent of CD30 employed according to the present invention may include derivatives of CD30. The derivatives of

1 CD30 may also include various structural forms of the primary protein, which  
2 retain biological activity. Due to the presence of ionizable amino and carboxyl  
3 groups, for example, a CD30 protein may be in the form of acidic or basic salts,  
4 or may be in neutral form. Individual amino acid may be modified by oxidation  
5 or reduction.

6 The primary structure of the CD30 protein according to the present  
7 invention may be modified by forming covalent or aggregative conjugates with  
8 other chemical moieties, such as glycosyl groups, lipids, phosphate, acetyl  
9 groups and the like, or by creating amino acid sequence mutants. Covalent  
10 derivatives are prepared by linking particular functional groups to CD30 amino  
11 acid side chains or at the N- or C-termini. Other derivatives of CD30 include  
12 covalent or aggregative conjugates of CD30 or its fragments with other proteins  
13 or polypeptides, such as by synthesis in recombinant culture as N-terminal or  
14 C-terminal fusions. For example, the conjugated peptide may be a signal (or  
15 leader) polypeptide sequence at the N-terminal region of the protein. The signal  
16 peptide co-translationally or post-translationally directs transfer of the protein  
17 from its site of synthesis to its site of function inside or outside of the cell  
18 membrane or wall (e.g., the yeast alpha-factor leader). The CD30 protein  
19 fusions can comprise peptides added to facilitate purification or identification  
20 of CD30 (e.g., poly-His or Fc domain of immunoglobulin molecule). The  
21 amino acid sequence of CD30 can also be linked to the peptide Asp-Tyr-Lys-  
22 Asp-Asp-Asp-Asp-Lys (DYKDDDDK) (Hopp et al., *Bio/Technology*, 6:1204,  
23 1988.) The latter sequence is highly antigenic and provides an epitope  
24 reversibly bound by a specific monoclonal antibody, enabling rapid assay and

1 facile purification of expressed recombinant protein. This sequence is also  
2 specifically cleaved by bovine mucosal enterokinase at the residue immediately  
3 following the Asp-Lys pairing.

4 CD30 with or without associated native-pattern glycosylation may also  
5 be used. CD30 expressed in yeast or mammalian expression systems, e.g.,  
6 COS-7 cells, may be similar or slightly different in molecular weight and  
7 glycosylation pattern than the native molecules, depending upon the expression  
8 system. Expression of CD30 DNAs in bacteria such as *E. coli* provides non-  
9 glycosylated molecules.

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#### 11 Mutant of CD30

12 A biologically functional equivalent of CD30 employed according to  
13 the present invention may include mutants of CD30. A CD30 mutant, as  
14 referred to herein, is a polypeptide homologous to CD30 but which has an  
15 amino acid sequence different from native CD30 because of a deletion,  
16 insertion or substitution.

17 The CD30 mutant may be constructed by, for example, making various  
18 substitutions of residues or sequences or deleting terminal or internal residues  
19 or sequences not needed for biological activity. For example, cysteine residues  
20 can be deleted or replaced with other amino acids to prevent formation of  
21 unnecessary or incorrect intramolecular disulfide bridges upon renaturation.  
22 Other approaches to mutagenesis involve modification of adjacent dibasic  
23 amino acid residues to enhance expression in yeast systems in which KEX2  
24 protease activity is present. Generally, substitutions should be made

1 conservatively; i.e., the most preferred substitute amino acids are those having  
2 physiochemical characteristics resembling those of the residue to be replaced.  
3 Similarly, when a deletion or insertion strategy is adopted, the potential effect  
4 of the deletion or insertion on biological activity should be considered.  
5 Substantially similar polypeptide sequences, as defined above, generally  
6 comprise a like number of amino acid sequences. To preserve the biological  
7 activity of CD30 proteins, deletions and substitutions will preferably result in  
8 homologous or conservatively substituted sequences, meaning that a given  
9 amino acid residue is replaced by a biologically similar residue. Examples of  
10 conservative substitutions include substitution of one aliphatic residue for  
11 another, such as Ile, Val, Leu, or Ala for one another, or substitutions of one  
12 polar residue for another, such as between Lys and Arg; Glu and Asp; or Gln  
13 and Asn. Other such conservative substitutions, for example, substitutions of  
14 entire regions having similar hydrophobicity characteristics, are well known.  
15 Moreover, particular amino acid differences between human, murine and other  
16 mammalian CD30 is suggestive of additional conservative substitutions that  
17 may be made without altering the essential biological characteristics of CD30.

18         The CD30 mutant may be constructed by deleting terminal or internal  
19 residues or sequences. Particularly preferred sequences include those in which  
20 the transmembrane region and intracellular domain of CD30 are deleted or  
21 substituted with hydrophilic residues to facilitate secretion of the receptor into  
22 the cell culture medium. The resulting protein is referred to as a soluble CD30  
23 mutant that retains its ability to bind to CD30 ligand. A particularly preferred  
24 soluble CD30 mutant construct is one that comprises the entire extracellular

1 region of CD30 terminating with Lys immediately adjacent to the  
2 transmembrane region. Additional amino acids may be deleted from the  
3 extracellular region of CD30, while retaining CD30 ligand binding activity.  
4 The present invention contemplates use of such soluble CD30 mutant  
5 constructs corresponding to all or part of the extracellular region of CD30.  
6 Other C-terminal deletions may be made as a matter of convenience by cutting  
7 CD30 cDNA with appropriate restriction enzymes and, if necessary,  
8 reconstructing specific sequences with synthetic oligonucleotide linkers.  
9 Soluble CD30 mutant with N-terminal deletions may also be used in the  
10 present invention. The resulting soluble CD30 mutant constructs are then  
11 inserted and expressed in appropriate expression vectors and assayed for the  
12 ability to bind to CD30 ligands.

13 Mutations in nucleotide sequences which are constructed for  
14 expression of the biologically functional equivalent of CD30, must preserve the  
15 reading frame phase of the coding sequences and preferably will not create  
16 complementary regions that could hybridize to produce secondary mRNA  
17 structures such as loops or hairpins that would adversely affect translation of  
18 the protein mRNA. Although a mutation site may be predetermined,  
19 predetermining the nature of the mutation per se is not necessary. For example,  
20 to select optimum characteristics of mutants at a given site, random  
21 mutagenesis may be conducted at the target codon and the expressed CD30  
22 mutants screened for the desired activity.

23 Not all mutations in the nucleotide sequence encoding a biologically  
24 functional equivalent of CD30 will be expressed in the final product. For

1 example, nucleotide substitutions may be made to enhance expression,  
2 primarily to avoid secondary structure loops in the transcribed mRNA or to  
3 provide codons that are more readily translated by the selected host, e.g., the  
4 well-known *E. coli* preference codons for *E. coli* expression.

5 Mutations can be introduced at particular loci flanked by restriction  
6 sites enabling ligation to fragments of the native sequence by synthesizing  
7 oligonucleotides containing a mutant sequence. Following ligation, the  
8 resulting reconstructed sequence encodes a biologically functional equivalent  
9 of CD30 having the desired amino acid insertion, substitution or deletion.

10 Alternatively, oligonucleotide-directed site-specific mutagenesis  
11 procedures can be employed to provide an altered gene having particular  
12 codons altered according to the substitution, deletion or insertion required.  
13 Exemplary methods of making the alterations set forth above are disclosed by  
14 Walder et al. (*Gene*, 42:133, 1986); Bauer et al. (*Gene*, 37:73, 1985); Craik  
15 (*BioTechniques*, Jan. 1985, 12-19); Smith et al. (*Genetic Engineering:*  
16 *Principles and Methods*, Plenum Press, 1981); and U.S. Pat. Nos. 4,518,584  
17 and 4,737,462.

#### 18 19 Chimeric protein

20 A biologically functional equivalent of CD30 employed according to  
21 the present invention may include a recombinant chimeric antibody molecule  
22 having a part of CD30 sequences substituted for the variable domains of either  
23 or both of the immunoglobulin molecule heavy and light chains and having  
24 unmodified constant region domains. For example, chimeric CD30/IgG may be

1 produced from two chimeric genes; a CD30/human kappa light chain chimera  
2 and a CD30/human gamma heavy chain chimera. Following transcription and  
3 translation of the two chimeric genes, the gene products assemble into a single  
4 chimeric antibody molecule having CD30 displayed tetravalently. The gene for  
5 CD30/human gamma heavy chain chimera alone may be used to produce a  
6 single chimeric antibody molecule having CD30 displayed bivalently. Such  
7 polyvalent forms of CD30 may have enhanced binding affinity for CD30 ligand.  
8 Additional details relating to the construction of such chimeric antibody  
9 molecules are disclosed in WO 89/09622 and EP 315062.

#### 11 Monovalent Form and Polyvalent Form of CD30 or a Biologically 12 Functional Equivalent of CD30

13 Both monovalent forms and polyvalent forms of CD30 or a  
14 biologically functional equivalent of CD30 may also be used in the present  
15 invention. Polyvalent forms possess multiple CD30 ligand binding sites. For  
16 example, a bivalent soluble CD30 may consist of two tandem repeats of amino  
17 acids corresponding to the extracellular domain of CD30, separated by a linker  
18 region. Alternate polyvalent forms may also be constructed, for example, by  
19 chemically coupling CD30 or the biologically functional equivalent of CD30 to  
20 any clinically acceptable carrier molecule, a polymer selected from the group  
21 consisting of Ficoll, polyethylene glycol or dextran using conventional  
22 coupling techniques. Alternatively, CD30 or the biologically functional  
23 equivalent of CD30 may be chemically coupled to biotin, and the biotin-CD30  
24 or biotin- biologically functional equivalent of CD30 conjugate then allowed to

1 bind to avidin, resulting in tetravalent avidin/biotin/CD30 or biologically  
2 functional equivalent of CD30 molecules. CD30 or the biologically functional  
3 equivalent of CD30 may also be covalently coupled to dinitrophenol (DNP) or  
4 trinitrophenol (TNP) and the resulting conjugate precipitated with anti-DNP or  
5 anti-TNP-IgM, to form decameric conjugates with a valency of 10 for CD30  
6 ligand binding sites.

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## 8 Expression of CD30

9 Recombinant expression vectors are preferably used to amplify or  
10 express DNA encoding CD30 or the biologically functional equivalent of  
11 CD30 to obtain purified CD30 or the biologically functional equivalent of  
12 CD30. Recombinant expression vectors are replicable DNA constructs which  
13 have synthetic or cDNA-derived DNA fragments encoding mammalian CD30  
14 or the biologically functional equivalent of CD30 operably linked to suitable  
15 transcriptional or translational regulatory elements derived from mammalian,  
16 microbial, viral or insect genes. A transcriptional unit generally comprises an  
17 assembly of (1) a genetic element or elements having a regulatory role in gene  
18 expression, for example, transcriptional promoters or enhancers, (2) a structural  
19 or coding sequence transcribed into mRNA and translated into protein, and (3)  
20 appropriate transcription and translation initiation and termination sequences,  
21 as described in detail below. Such regulatory elements may include an operator  
22 sequence to control transcription, a sequence encoding suitable mRNA  
23 ribosomal binding sites. The ability to replicate in a host, usually conferred by  
24 an origin of replication and a selection gene to facilitate recognition of

1 transformants may additionally be incorporated. DNA regions are operably  
2 linked when they are functionally related to each other. For example, DNA for  
3 a signal peptide (secretory leader) is operably linked to DNA for a polypeptide  
4 if it is expressed as a precursor that participates in the secretion of the  
5 polypeptide. A promoter is operably linked to a coding sequence if it controls  
6 the transcription of the sequence. A ribosome-binding site is operably linked to  
7 a coding sequence if it is positioned to permit translation. Generally, “operably  
8 linked” means contiguous and, in the case of secretory leaders, in a reading  
9 frame. Structural elements intended for use in yeast expression systems  
10 preferably include a leader sequence enabling extracellular secretion of  
11 translated protein by a host cell. Alternatively, where recombinant protein is  
12 expressed without a leader or transport sequence, the recombinant protein may  
13 include an N-terminal methionine residue. This residue may optionally be  
14 subsequently cleaved from the expressed recombinant protein to provide a final  
15 product.

16 DNA sequences encoding mammalian CD30 proteins that are to be  
17 expressed in a cell will preferably contain no introns that could prematurely  
18 terminate transcription of DNA into mRNA. However, premature termination  
19 of transcription may be desirable, for example, where it would result in mutants  
20 having advantageous C-terminal truncations, for example, deletion of a  
21 transmembrane region to yield a soluble receptor not bound to the cell  
22 membrane. Due to code degeneracy, there can be considerable variation in  
23 nucleotide sequences encoding the same amino acid sequence. Other  
24 embodiments include sequences capable of hybridizing to the sequences of the

1 provided cDNA under moderately stringent conditions and other sequences  
2 hybridizing or degenerate to those which encode biologically active CD30  
3 polypeptides.

4           Recombinant CD30 DNA is expressed or amplified in a recombinant  
5 expression system, comprising a substantially homogeneous monoculture of  
6 suitable host microorganisms or cells, that have stably integrated (by  
7 transformation or transfection) a recombinant transcriptional unit into  
8 chromosomal DNA or carry the recombinant transcriptional unit as a  
9 component of a resident plasmid. Generally, cells constituting the system are  
10 the progeny of a single ancestral transformant. Recombinant expression  
11 systems as defined herein will express heterologous protein upon induction of  
12 the regulatory elements linked to the DNA sequence or synthetic gene to be  
13 expressed.

14           Transformed host cells have been transformed or transfected with  
15 CD30 vectors constructed using recombinant DNA techniques. Transformed  
16 host cells ordinarily express CD30, but host cells transformed for purposes of  
17 cloning or amplifying CD30 DNA do not need to express CD30. Expressed  
18 CD30 will be deposited in the cell membrane or secreted into the culture  
19 supernatant, depending on the CD30 DNA selected. Suitable host cells for  
20 expression of mammalian CD30 include prokaryotes, yeast, insect cells or  
21 higher eukaryotic cells under the control of appropriate promoters. Prokaryotes  
22 include gram negative or gram positive organisms, for example *E. coli* or  
23 *bacilli*. Higher eukaryotic cells include established cell lines of mammalian  
24 origin as described below. Cell-free translation systems could also be employed

1 to produce mammalian CD30 using RNAs derived from the DNA constructs of  
2 the present invention. Appropriate cloning and expression vectors for use with  
3 bacterial, fungal, yeast, and mammalian cellular hosts are described by Pouwels  
4 et al. (Cloning Vectors: A Laboratory Manual, Elsevier, N.Y., 1985).

5 Prokaryotic expression hosts may be used for expression of CD30 that  
6 do not require extensive proteolytic and disulfide processing. Prokaryotic  
7 expression vectors generally comprise one or more phenotypic selectable  
8 markers, for example a gene encoding proteins conferring antibiotic resistance  
9 or supplying an autotrophic requirement and an origin of replication recognized  
10 by the host to ensure amplification within the host. Suitable prokaryotic hosts  
11 for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium*,  
12 and various species within the genera *Pseudomonas*, *Streptomyces*, and  
13 *Staphylococcus*, although others may also be employed as a matter of choice.

14 Useful expression vectors for bacterial use can comprise a selectable  
15 marker and bacterial origin of replication derived from commercially available  
16 plasmids comprising genetic elements of the well-known cloning vector  
17 pBR322 (ATCC 37017). Such commercial vectors include, for example,  
18 pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and pGEM1  
19 (Promega Biotec, Madison, Wis. U.S.A.). These pBR322 "backbone" sections  
20 are combined with an appropriate promoter and the structural sequence to be  
21 expressed. *E. coli* is typically transformed using derivatives of pBR322, a  
22 plasmid derived from an *E. coli* species (Bolivar et al., *Gene*, 2:95, 1977).  
23 pBR322 contains genes for ampicillin and tetracycline resistance and thus  
24 provides simple means for identifying transformed cells.

1 Promoters commonly used in recombinant microbial expression  
2 vectors include the beta-lactamase (penicillinase) and lactose promoter system  
3 (Chang et al., *Nature*, 275:615, 1978; and Goeddel et al., *Nature*, 281:544,  
4 1979), the tryptophan (trp) promoter system (Goeddel et al., *Nucl. Acids Res.*,  
5 8:4057, 1980; and EPA, 36, 776) and tac promoter (Maniatis, *Molecular*  
6 *Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, p. 412, 1982).

7 Recombinant CD30 proteins may also be expressed in yeast hosts,  
8 preferably from the *Saccharomyces* species, such as *S. cerevisiae*. Yeast of  
9 other genera, such as *Pichia* or *Kluyveromyces* may also be employed.

10 Various mammalian or insect cell culture systems are also  
11 advantageously employed to express recombinant protein. Expression of  
12 recombinant proteins in mammalian cells is particularly preferred because such  
13 proteins are generally correctly folded, appropriately modified and completely  
14 functional. Examples of suitable mammalian host cell lines include the COS-7  
15 lines of monkey kidney cells, described by Gluzman (*Cell*, 23:175, 1981), and  
16 other cell lines capable of expressing an appropriate vector including, for  
17 example, L cells, C127, 3T3, Chinese hamster ovary (CHO), HeLa and BHK  
18 cell lines. Mammalian expression vectors may comprise nontranscribed  
19 elements such as an origin of replication, a suitable promoter and a suitable  
20 enhancer linked to the gene to be expressed, and other 5' or 3' flanking  
21 nontranscribed sequences, and 5' or 3' nontranslated sequences, such as  
22 necessary ribosome-binding sites, a polyadenylation site, splice donor and  
23 acceptor sites, and transcriptional termination sequences. Baculovirus systems  
24 for production of heterologous proteins in insect cells are reviewed by Luckow

1 and Summers, *Bio/Technology*, 6:47 (1988).

2       The transcriptional and translational control sequences in expression  
3 vectors to be used in transforming vertebrate cells may be provided by viral  
4 sources. For example, commonly used promoters and enhancers are derived  
5 from Polyoma, Adenovirus 2, Simian Virus 40 (SV40) and human  
6 cytomegalovirus. DNA sequences derived from the SV40 viral genome, for  
7 example, SV40 origin, early and late promoter, enhancer, splice and  
8 polyadenylation sites may be used to provide the other genetic elements  
9 required for expression of a heterologous DNA sequence. The early and late  
10 promoters are particularly useful because both are obtained easily from the  
11 virus as a fragment that also contains the SV40 viral origin of replication (Fiers  
12 et al., *Nature*, 273:113, 1978). Smaller or larger SV40 fragments may also be  
13 used, provided the approximately 250 bp sequence extending from the *HindIII*  
14 site toward the *BglII* site located in the viral origin of replication is included.  
15 Further, mammalian genomic CD30 promoter, control and/or signal sequences  
16 may be utilized, provided such control sequences are compatible with the host  
17 cell chosen. Additional details regarding the use of a mammalian high  
18 expression vector to produce a recombinant mammalian CD30 are provided in  
19 examples below. A useful system for stably high level expression of  
20 mammalian receptor cDNAs in C127 murine mammary epithelial cells can be  
21 constructed substantially as described by Cosman et al. (*Mol. Immunol.*, 23:935,  
22 1986).

23       Recombinant expression vectors comprising CD30 cDNAs are stably  
24 integrated into a host cell's DNA. Elevated levels of expression product are

1 achieved by selecting cell lines having amplified numbers of vector DNA. Cell  
2 lines having amplified numbers of vector DNA are selected, for example, by  
3 transforming a host cell with a vector comprising a DNA sequence encoding an  
4 enzyme that is inhibited by a known drug. The vector may also comprise a  
5 DNA sequence encoding a desired protein. Alternatively, the host cell may be  
6 co-transformed with a second vector comprising the DNA sequence encoding  
7 the desired protein. The transformed or co-transformed host cells are then  
8 cultured in increasing concentrations of the known drug, thereby selecting  
9 drug-resistant cells. Such drug-resistant cells survive in increased  
10 concentrations of the toxic drug by over-production of the enzyme that is  
11 inhibited by the drug, frequently as a result of amplification of the gene  
12 encoding the enzyme. Where drug resistance is caused by an increase in the  
13 copy number of the vector DNA encoding the inhibitable enzyme, there is a  
14 concomitant co-amplification of the vector DNA encoding the desired protein  
15 in the host cell's DNA.

16 A preferred system for such co-amplification uses the gene for  
17 dihydrofolate reductase (DHFR), which can be inhibited by the drug  
18 methotrexate (MTX). To achieve co-amplification, a host cell that lacks an  
19 active gene encoding DHFR is either transformed with a vector that comprises  
20 DNA sequence encoding DHFR and a desired protein or is co-transformed with  
21 a vector comprising a DNA sequence encoding DHFR and a vector comprising  
22 a DNA sequence encoding the desired protein. The transformed or co-  
23 transformed host cells are cultured in media containing increasing levels of  
24 MTX, and those cells lines that survive are selected.

1           A particularly preferred co-amplification system uses the gene for  
2 glutamine synthetase (GS), which is responsible for the synthesis of glutamate  
3 and ammonia using the hydrolysis of ATP to ADP and phosphate to drive the  
4 reaction. GS is subject to inhibition by a variety of inhibitors, for example  
5 methionine sulfoximine (MSX). Thus, CD30 can be expressed in high  
6 concentrations by cells transformed with a vector comprising the DNA  
7 sequence for GS and CD30, or co-transformed with a vector comprising a DNA  
8 sequence encoding GS and a vector comprising a DNA sequence encoding  
9 CD30. These cells are selected by culturing the host cells in media containing  
10 increasing levels of MSX and selecting surviving cells. The GS co-  
11 amplification system, appropriate recombinant expression vectors and cells  
12 lines are described in the PCT applications WO 87/04462, WO 89/01036, WO  
13 89/10404 and WO 86/05807.

14           A preferred eukaryotic vector for expression of CD30 DNA is  
15 disclosed in the examples that follow. This vector, referred to as pMIB,  
16 contains regulatory sequences from honeybee.

17

#### 18 Purification of CD30

19           Purified mammalian CD30 or the biological functional equivalent are  
20 prepared by culturing suitable host/vector systems to express the recombinant  
21 translation products, which are then purified from culture media or cell  
22 extracts.

23           For example, supernatants from systems that secrete recombinant  
24 protein into culture media can be first concentrated using a commercially

1 available protein concentration filter, for example, an Amicon or Millipore  
2 ultrafiltration unit. Following the concentration step, the concentrate can be  
3 applied to a suitable purification matrix. A suitable affinity matrix can  
4 comprise an antibody molecule bound to a suitable support. Alternatively, an  
5 anion exchange resin can be employed, for example, a matrix or substrate  
6 having pendant diethylaminoethyl (DEAE) groups. The matrices can be  
7 acrylamide, agarose, dextran, cellulose or other types commonly employed in  
8 protein purification. Alternatively, a cation exchange step can be employed.  
9 Suitable cation exchangers include various insoluble matrices comprising  
10 sulfopropyl or carboxymethyl groups. Sulfopropyl groups are preferred.

11 Finally, one or more reversed-phase high performance liquid  
12 chromatography (RP-HPLC) steps employing hydrophobic RP-HPLC media,  
13 e.g., silica gel having pendant methyl or other aliphatic groups, can be  
14 employed to further purify a CD30 composition. Some or all of the foregoing  
15 purification steps, in various combinations, can also be employed to provide a  
16 homogeneous recombinant protein.

17 Recombinant protein produced in bacterial culture is usually isolated  
18 by initial extraction from cell pellets, followed by one or more concentration,  
19 salting-out, aqueous ion exchange or size exclusion chromatography steps.  
20 Finally, high performance liquid chromatography (HPLC) can be employed for  
21 final purification steps. Microbial cells employed in expression of recombinant  
22 mammalian CD30 can be disrupted by any convenient method, including  
23 freeze-thaw cycling, sonication, mechanical disruption or use of cell lysing  
24 agents.

1            Fermentation of yeast that expresses mammalian CD30 as a secreted  
2    protein greatly simplifies purification. Secreted recombinant protein resulting  
3    from large-scale fermentation can be purified by methods analogous to those  
4    disclosed by Urdal et al. (*J. Chromatog.*, 296:171, 1984). This reference  
5    describes two sequential, reversed-phase HPLC steps for purification of  
6    recombinant human GM-CSF on a preparative HPLC column.

7            Human CD30 synthesized in recombinant culture is characterized by  
8    the presence of non-human cell components, including proteins, in amounts  
9    and of a character that depend upon the purification steps taken to recover  
10   human CD30 from the culture. These components ordinarily will be of yeast,  
11   prokaryotic or non-human higher eukaryotic origin and preferably are present  
12   in innocuous contaminant quantities, on the order of less than about 1 percent  
13   by weight. Further, recombinant cell culture enables the production of CD30  
14   free of proteins which may be normally associated with CD30 as it is found in  
15   nature in its species of origin.

16

#### 17    The Effect of Soluble CD30 or Plate-bound CD30 on T Cells

18            The present invention provides a method of lowering the levels of T  
19   cell proliferation and activation in activated human T cells, comprising  
20   administering an effective amount of CD30 or a biologically functional  
21   equivalent thereof. Preferably, the biologically functional equivalent thereof is  
22   a chimeric protein of CD30, such as CD30-Fc. More preferably, the  
23   biologically functional equivalent is a chimeric antibody comprising an  
24   extracellular domain of CD30 fused to an immunoglobulin heavy chain

1 constant region polypeptide. Anti-CD3 treatment of T cells is considered an in-  
2 vitro method to mimic T cell activation in vivo. Tritium thymidine  
3 incorporation is the method employed in the present invention to measure the  
4 proliferation responses of activated T cells. The amount of IL-2 secreted by  
5 activated T cells into the medium was also measured. IL-2 is the key cytokine  
6 responsible for the proliferative responses after T cell activation, and can play  
7 in either an autocrine or a paracrine manner. The CD30 or the biologically  
8 functional equivalent thereof is able to decrease the production of IL-2 from  
9 anti-CD3-treated T cells. To measure the levels of T cell activation, the amount  
10 of CD25 and CD26 expression in activated T cells was measured. Both of these  
11 surface markers are known to express after adequate T cell activation in vitro or  
12 in vivo. The decrease in the levels of T-cell proliferation by a therapeutically  
13 effective amount of CD30 or a biologically functional equivalent thereof can be  
14 attenuated with IL-2 supplementation.

15 In summary, the present invention established that CD30 is involved in  
16 the inhibition of T-cell proliferation, IL-2 production, and expression of CD25  
17 and CD26 by T cells. Controlling T-cell proliferation or T-cell activation could  
18 be a method for treating immune disorders.

19 The present invention provides a method for treating immune disorders  
20 comprising administering CD30 or a biologically functional equivalent thereof  
21 to a human afflicted with immune disorders. The CD30 or the biologically  
22 functional equivalent thereof can be administered intravenously and be  
23 administered in the form of a composition that additionally comprises a diluent,  
24 excipient or carrier. The biologically functional equivalent is a soluble chimeric

1 protein. The immune disorders are associated with T-cell activation, T-cell  
2 proliferation, T-cell production of IL-2, T-cell expression of CD25 or CD26.

3 The present invention also provides a pharmaceutical composition for  
4 treating immune disorders in a human comprising a therapeutically effective  
5 amount of CD30 or a biologically functional equivalent thereof, and a  
6 pharmaceutically acceptable carrier, excipient or diluent.

7 The pharmaceutical composition is prepared by known procedures  
8 using well-known and readily available ingredients. The compositions of this  
9 invention may be formulated to provide quick, sustained or delayed release of  
10 the active ingredient after administration to the patient by employing  
11 procedures well-known in the art. In making the compositions of the present  
12 invention, the active ingredient will usually be admixed with a carrier, diluent  
13 or excipient. The carrier may be in the form of a capsule, sachet, paper or other  
14 container. The diluent may be a solid, semi-solid or liquid material that acts as  
15 a vehicle, excipient or medium for the active ingredient. The excipient may be  
16 starch, sucrose, starch, glycol, mannitol, microcrystalline cellulose and so on.

17 All of the documents or publications recited in the text are incorporated  
18 herein by reference.

19

20 The following examples are offered by way of illustration, and not by  
21 way of limitation.

22

23 EXAMPLES

24

1    Example 1

2    Construction and Expression of Soluble Human CD30-Fc Fusion Protein

3

4            The cloning of the cDNA for the human CD30 has been described in  
5    detail (Durkop et al., *Cell*, 68:421, 1992).

6            With reference to Fig. 1 and 2, the gene segment for the CD30-Fc  
7    fusion protein was transferred to the MIB vector (Invitrogen, San Diego, CA).  
8    The primary translational product of the plasmid coding for rhu (recombinant  
9    human) CD30-Fc is a single molecule of soluble CD30 (corresponding to the  
10   extracellular domain of the protein) linked to a single chain of Fc derived from  
11   human IgG1. Following translation, this fusion molecule dimerizes via cysteine  
12   residues in the Fc region to form dimeric rhu CD30-Fc. The gene segment for  
13   rhu CD30-Fc is inserted into pMIB, a commercially available plasmid for  
14   expression of a desired protein in insect cells.

15           The rhu CD30-Fc fusion protein was created by ligating the following  
16   fragments (a) and (b).

17           (a) The first segment contains the protein segment for the extracellular  
18   domain of CD30. The corresponding gene segment was generated by PCR by  
19   use of the sense oligonucleotides 5'-AAGAATTCTTCCCACAGGATCGAC  
20   and the anti-sense oligonucleotides 5'-  
21   TTGTTAACCTTCCCCGTGGAGGAGAG.

22           (b) The other protein segment contains a 238 amino acid polypeptide,  
23   derived from a 715 bp fragment from human IgG1, coding for the Fc portion of  
24   human IgG1.

1           The pMIB containing the gene for rhu CD30-Fc was transfected into  
2   the insect cells Sf21. High producers were selected with ELISA kits for the  
3   human immunoglobulin Fc portion. Purification was done with protein A  
4   columns (Amersham Pharmacia, Uppsala, Sweden). Bound antibodies were  
5   eluted with a buffer containing 0.1 mol/L glycine, 0.15 mol/L NaCl, pH 2.4 and  
6   brought to neutral pH with 0.5 mol/L sodium phosphate, pH 8.0. Purified  
7   proteins were dialyzed extensively with PBS (phosphate buffered saline) and  
8   sterilized by filtration. The concentrations of proteins were determined by a  
9   bicinchoninic acid-based protein assay (Pierce, Rockford, IL).

10          Transfections were performed by mixing pMIB/rhuCD30-Fc plasmid  
11   DNA with Lipofectin from Gibco BRL. Approximately 10µg of DNA was  
12   added to 10 cm petri dishes containing Sf21 cells. After the initial transfection,  
13   cells were selected in a selective medium containing 50µg/ml Blasticidin. The  
14   resulting colonies were then transferred to 24 well plates and analyzed for rhu  
15   CD30-Fc expression. The highest expressing cultures were subjected to  
16   amplification by exposure to increasing concentrations of Blasticidin. Cells  
17   able to grow were cloned by limiting dilution in 96 well plates. The highest  
18   expressing clones were transferred to a suspension culture, and the final  
19   selection of clones were made based on its high level of rhu CD30-Fc  
20   expression under these conditions.

21

## 22   Example 2

### 23   Effect of CD30-Fc on T cell proliferation

24

1           Total peripheral blood mononuclear cells (PBMCs) were separated on  
2   Ficoll-Hypaque (Amersham Biosciences, Uppsala, Sweden). CD3<sup>+</sup> and CD4<sup>+</sup>  
3   T cells in PBMCs were affinity-purified by use of the CD3<sup>+</sup> and CD4<sup>+</sup> T  
4   MACS isolation kits respectively (Miltenyi Biotech, Bergisch Gladbach,  
5   Germany) with a negative selection method. The resulting purified cells were  
6   more than 95% positive for CD3 or CD4. The retained cells in the column were  
7   eluted outside the magnetic field, treated with 2% paraformaldehyde (J.T.  
8   Baker, Phillipsburg, NJ) for two hours, and washed extensively with PBS.  
9   These cells were used as accessory cells in the T-cell proliferation assay.

10           To study the effect of the CD30-Fc fusion protein on T-cell  
11   proliferation, plate-bound and soluble CD30-Fc fusion protein was used. Plates  
12   with different concentrations of CD30-Fc chimeric protein or human IgG  
13   (Cappel, Aurora, OH) were stored at 4°C overnight and washed with PBS  
14   before use. T cells and accessory cells (each 1x10<sup>5</sup> cells/well) were treated with  
15   soluble anti-CD3 (HIT3a, BD PharMingen, San Diego, CA) at 0.5 µg/ml and  
16   different concentrations of CD30-Fc fusion protein or human IgG and were  
17   incubated in triplicate in U-bottom 96-well plates at 37°C/5% CO<sub>2</sub> for 3 days.  
18   During the last 6 hours of incubation, cultures were pulsed with 0.5 µCi/well  
19   [<sup>3</sup>H]thymidine. Cells were then harvested with a Packard Cell Harvester onto  
20   unifilter plates. Cell-associated radioactivity was measured by scintillation  
21   counting. In other studies, soluble CD30-Fc or IgG was substituted for plate-  
22   bound CD30-Fc or IgG.

23           As shown in Figure 3A, plate-bound CD30-Fc fusion protein had a  
24   dose-dependent inhibitory effect on the proliferation of anti-CD3-treated T

1 cells. A dramatic inhibition was observed when the concentrations were equal  
2 to or more than 10 µg/ml. CD30 inhibits anti-CD3-induced T-cell proliferation,  
3 while human IgG had little effect. In Fig. 3B, a similar dose-dependent  
4 inhibitory effect on T cell proliferation was observed, although higher  
5 concentrations were required to achieve a similar effect on the inhibition of T  
6 cell proliferation, when soluble CD30-Fc was substituted for plate-bound  
7 CD30-Fc.

8  
9

### 10 Example 3

11 Effect of CD30-Fc on IL-2 production from activated T cells.

12

13 To study the effect of CD30-Fc on IL-2 production from anti-CD3-  
14 treated T cells, IL-2 amounts in the culture supernatant were measured. T cells  
15 and accessory cells ( $1 \times 10^5$  cells/well for each type of cells) were co-cultured  
16 in triplicate in U-bottom 96-well plates with soluble anti-CD3 at 0.5 µg/ml and  
17 different concentrations of the plate-bound CD30-Fc chimeric protein. Plates  
18 were incubated at 37°C/5% CO<sub>2</sub> for 3 days. The supernatant was collected, and  
19 levels of IL-2 production were measured with an IL-2 ELISA Kit (Diaclone,  
20 France) according to the manufacturer's protocol.

21 IL-2 production from anti-CD3-treated T cells co-cultured with higher  
22 concentrations of CD30-Fc chimeric protein was not detectable (Fig. 4). CD30-  
23 Fc is able to inhibit the production of IL-2 from anti-CD3-treated T cells.

24

1     Example 4

2     Effect of IL-2 addition on the inhibition of T cell proliferation by CD30-Fc.

3

4             To study the effect of IL-2 addition on T-cell proliferation,  
5     recombinant human IL-2 (United States Biological, Swampscott, MA) at 1  
6     ng/ml was used. T cells, accessory cells ( $1 \times 10^5$  cells/well for each type of  
7     cells) and different concentrations of the plate-bound CD30-Fc chimeric  
8     protein were co-cultured in triplicate in U-bottom 96-well plates with soluble  
9     anti-CD3 at 0.5  $\mu$ g/ml and IL-2. Plates were incubated at 37°C/5% CO<sub>2</sub> for 3  
10    days. During the last 6 h of incubation, cultures were pulsed with 0.5  $\mu$ Ci/well  
11    of [3H]thymidine. Cells were then harvested with a Packard Cell Harvester  
12    onto unifilter plates. Cell-associated radioactivity was measured by scintillation  
13    counting.

14            Addition of exogenous IL-2 restored T-cell proliferation inhibited by  
15    CD30-Fc fusion protein (Fig. 5), supporting the claim that the decrease in the  
16    levels of T-cell proliferation by CD30 is attenuated with IL-2 supplementation.

17

18    Example 5

19    CD30-Fc Chimeric Protein Inhibit Expression of CD25 and CD26 by Anti-  
20    CD3-treated T Cells

21

22            T cells in the co-cultures with different concentrations of the plate-  
23    bound CD30-Fc chimeric protein or human IgG were analyzed for the  
24    expression of CD25 or CD26 with FACS (BD Immunocytometry Systems, San

1 Jose, CA). FITC-labeled CD3 and PE-labeled CD25/CD26 mouse monoclonal  
2 antibodies were used. For negative controls, FITC- and PE-labeled mouse IgGs  
3 were substituted. All antibodies were purchased from BD PharMingen. As  
4 shown in Fig. 6, CD30-Fc chimeric protein was able to inhibit the expression  
5 of CD25 and CD26 in anti-CD3-treated T cells. The X axis of the dot plots  
6 stand for CD3, and the Y-axis for CD25 or CD26.

7  
8 Although the invention has been explained in relation to its preferred  
9 embodiments, many other possible modifications and variations can be made  
10 without departing from the spirit and scope of the invention as hereinafter  
11 claimed.